PATENT
Docket No.: 20011/1381

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	: Beck et al.	Examiner: B. M. Robinson
Serial No.	: 09/902,845	Art Unit: 1625
Cnfrm. No.	: 9511	
Filed	: July 11, 2001	
For	: NOVEL 4-PHENYL SUBSTITUTED TETRAHYDROISOQUINOLINES AND THERAPEUTIC USE THEREOF	

DECLARATION OF BRUCE F. MOLINO UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box. 1450
Alexandria, Virginia 22313-1450

Dear Sir:

I, BRUCE F. MOLINO, pursuant to 37 C.F.R. § 1.132, declare:

1. I received a B.S. degree in Chemistry from Rutgers University in 1977, and a Ph.D. degree in Organic Chemistry from the University of Maryland in 1984.

2. I am currently the Senior Director of the Medicinal Chemistry Department at Albany Molecular Research, Inc. ("AMRI"), Albany, New York.

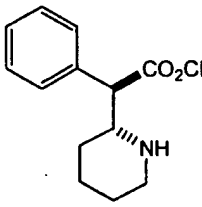
3. It is my understanding that AMRI is the assignee of the above-identified patent application.

4. I am presenting this declaration to provide experimental data to demonstrate that the compounds of the present invention are effective for treating an animal afflicted with a neurological or psychological disorder such as attention deficit-hyperactivity disorder, anxiety, depression, post-traumatic stress disorder, supranuclear palsy, feeding disorders, obsessive compulsive disorder, analgesia, smoking cessation, panic attacks, Parkinson's, or phobia (collectively referred to herein as "Neurological/Psychological Disorders").

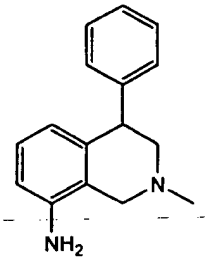
5. The following assays were used to analyze the biological activity and therapeutic efficacy of the compounds of the present invention: (i) a primary binding assay and (ii) a tetrabenazine ("TBZ") assay. The basic protocols for these assays are described in the present application on page 86, line 20 to page 88, line 31, and are reiterated below (as appropriate).

6. The primary binding assay was used to the determine activity of the compounds at three different human neurotransmitter transporters, i.e., at the norepinephrine, dopamine, and serotonin transporters. Clinically, it is well established in the field of central nervous system ("CNS") therapeutics that compounds with activity at the norepinephrine, dopamine, and serotonin transporters can be used in treating the Neurological/Psychological Disorders described in the present application. The TBZ assay, also known as the TBZ ptosis reversal assay, is an *in vivo* assay that can detect CNS penetration by a compound and can predict the efficacy of a compound in the treatment of depression. Based on the experimental data presented below, it is scientifically reasonable to conclude that the compounds of the present invention can be used in methods to treat animals afflicted with the Neurological/Psychological Disorders.

7. In addition to the compounds of the present invention (identified in **Tables 1** and **2** *infra* with reference to various examples in the present application and referred to herein as the "PH-7222 Compounds"), the following compounds were analyzed for their biological activity and potential efficacy for treating the Neurological/Psychological Disorders:



Ritalin® or *dl-threo*-methylphenidate
1



Nomifensine
2

8. In order to evaluate the relative affinity of the various compounds for the norepinephrine transporter ("NET"), the dopamine transporter ("DAT"), and the serotonin transporter ("SERT"), HEK293E cell lines were developed to express each of the three human transporters. cDNAs containing the complete coding regions of each transporter were amplified by polymerase chain reaction from human brain libraries. The cDNAs contained in pCRII vectors were sequenced to verify their identity and then subcloned into an Epstein-Barr virus-based expression plasmid (Shen et al., Gene 156:235-239 (1995)). This plasmid containing the coding sequence for one of the human transporters was transfected into HEK293E cells. Successful transfection was verified by the ability of known reuptake blockers to inhibit the uptake of tritiated norepinephrine, dopamine, or serotonin.

9. To test the compounds for binding, the transfected HEK293E cells were homogenized, centrifuged, and then resuspended in incubation buffer (50 mM Tris, 120 mM NaCl, 5 mM KCl, pH 7.4). The appropriate radioligand was then added, as follows: (i) for NET binding, [^{3}H] Nisoxetine (86.0 Ci/mmol, NEN/DuPont) was added to a final concentration of approximately 5 nM; (ii) for DAT binding, [^{3}H] WIN 35,428 (84.5 Ci/mmol) at 15 nM was added; and for SERT binding, [^{3}H] Citolapram (85.0 Ci/mmol) at 1 nM was added. Various concentrations of the compound of interest (i.e., ranging from 10^{-5} to 10^{-11} M) were then added to displace the radioligand. Incubation was carried out at room temperature for 1 hour in a 96-well plate. Following incubation, the plates were placed on a harvester and washed quickly 4 times with a buffer (50 mM tris, 0.9% NaCl, pH 7.4) where the cell membranes containing the bound radioactive label were trapped on Whatman GF/B filters. Scintillation cocktail was added to the filters which were then counted in a Packard TopCount. Binding affinities of the compounds of interest were determined by non-linear curve regression using GraphPad Prism 2.01 software. Non-specific binding was determined by displacement with 10 micromolar mazindol. The results of these binding assays for the various compounds tested are set forth in **Table 1** (below).

Table 1

PH-7222 Compounds Example # or Additional compounds	NET, Ki nM	DAT, Ki nM	SERT, Ki nM
Ritalin®	610	37	32000
Nomifensine	23	72	1036
Example 1	93	198	988
Example 16	25	111	8425
Example 18	10	31.5	2077
Example 26	23.5	55.5	443
Example 28	39	49	348
Example 33	14.5	21.5	36
Example 39	15	50	260
Example 42	25.5	28	1117
Example 45	13	4.7	125
Example 70	3.5	10.5	934
Example 72	12.5	13.5	2562
Example 74	6.1	14.6	365
Example 75	6.0	24.5	793
Example 77	13	12	140
Example 80	5.6	1.7	280
Example 81	43.5	64	1134
Example 91	31	207	796
Example 92	15.5	443	972
Example 93	15	222	588
Example 106	6.0	100	796
Example 108	116	814	3110
Example 123	637	626	1766

10. The results in **Table 1** demonstrate that the PH-7222 Compounds possess binding affinity (indicated as a "Ki" value) to each of the three human monoamine transporters (i.e., NET, DAT, and SERT) with varying potency and selectivity. It is well established in the field of human therapeutics that a compound's ability to selectively inhibit one or more of the monoamine transporters is indicative of that compound's efficacy as a therapeutic for the various Neurological/Psychological Disorders referenced in the present application. A higher Ki value for a compound indicates that the compound has less binding affinity for a target molecule (e.g., a protein such as NET, DAT, or SERT) than is so for a different compound with a lower Ki for the same target molecule. Conversely, lower Ki values are indicative of greater binding affinities.

11. There are a number of marketed drugs that work by selective inhibition of reuptake of monoamines by one or more of the monoamine transporters. For example, the

clinically approved drug Ritalin®, is useful for the treatment of attention deficit hyperactivity disorder ("ADHD") in adults and children. Mechanistically, Ritalin® is believed to work by blocking predominantly dopamine uptake by the dopamine transporter (i.e., DAT). Zyban® (i.e., Bupropion), which has been approved for smoking cessation, works by blocking norepinephrine and dopamine uptake at the NET and DAT. Further, many clinically used antidepressants (e.g., Prozac®, Zoloft®, Paxil®, and others) work by blocking transport of serotonin at the SERT. More recently approved drugs like Cymbalta® (Duloxetine) and Effexor® (Venlafaxine) work by blocking both norepinephrine and serotonin reuptake at the NET and the SERT. Still other CNS agents, like Brasofensine (NS-2214) and BTS 74 398, are selective dopamine reuptake inhibitors (DAT) that are under investigation for treatment of Parkinson's disease. Therefore, knowledge of the *in vitro* activity for blocking monoamine reuptake at the NET, DAT, and SERT is a sound basis for determining clinical utility.

12. The results in **Table 1** demonstrate that Ritalin® is generally between about 4-fold and 173-fold less potent for NET (based on the NET, Ki value) than the PH-7222 Compounds (with the exception of the PH-7222 Compound of Example 123). Regarding DAT, **Table 1** demonstrates that Ritalin®: (i) has a comparable potency for DAT (based on the DAT, Ki value) to that of certain PH-7222 Compounds (Examples 18, 26, 28, 33, 39, 42, and 75); (ii) is generally between about 2-fold and 21-fold less potent for DAT (based on the DAT, Ki value) than a number of the PH-7222 Compounds (Examples 45, 70, 72, 74, 77, and 80); and (iii) is between about 1-fold and 21-fold more potent for DAT than a number of other PH-7222 Compounds (e.g., Examples 1, 16, 81, 91, 92, 93, 106, 108, and 123). As to SERT, **Table 1** demonstrates that Ritalin® is generally between about 3-fold and 888-fold less potent for SERT (based on the SERT, Ki value) than the PH-7222 Compounds.

13. The results in **Table 1** further demonstrate that Nomifensine: (i) has a comparable potency for NET (based on the NET, Ki value) to that of certain of the PH-7222 Compounds (Examples 16, 26, 33, 39, 42, 45, 72, 77, 91, 92, and 93); (ii) is between about 1-fold and 6-fold less potent for NET than certain of the PH-7222 Compounds (Examples 18, 70, 74, 75, 80, and 106); and (iii) is between about 1-fold and 27-fold more potent for NET than certain other PH-7222 Compounds (Examples 1, 28, 81, 108, and 123). Regarding DAT, **Table 1** demonstrates that Nomifensine: (i) has a comparable potency for DAT (based on the DAT, Ki value) to that of certain of the PH-7222 Compounds (Examples 26, 28, 39, 81, and 106); (ii) is generally between about 1-fold and 41-fold less potent for DAT than certain of the PH-7222 Compounds (Examples 18, 33, 42, 45, 70, 72, 74, 75, 77, and 80); and (iii) is generally between about 1-fold and 10-fold more potent for DAT than certain of the

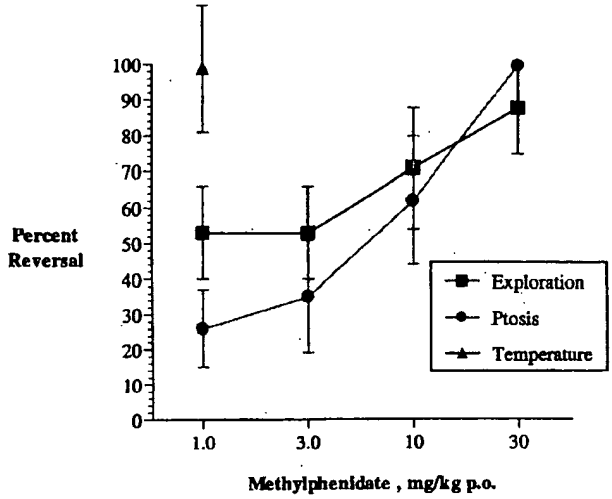
other PH-7222 Compounds (Examples 1, 16, 91, 92, 93, 108, and 123). As to SERT, **Table 1** shows that Nomifensine: (i) has a comparable potency for SERT (based on the SERT, Ki value) to that of certain of the PH-7222 Compounds (Examples 1, 42, 70, 75, 81, 91, 92, 93, and 106); (ii) is between about 1-fold and 28-fold less potent for SERT than certain of the PH-7222 Compounds (Examples 26, 28, 33, 39, 45, 74, 77, and 80); and (iii) is between about 1-fold and 7-fold more potent for SERT than certain other PH-7222 Compounds (Examples 16, 18, 72, 108, and 123).

14. Using the TBZ assay, in order to assess *in vivo* activity of the PH-7222 Compounds, such compounds were tested for their ability to reverse TBZ effects after oral administration to mice. The activity of the PH-7222 Compounds was compared with two standard CNS agents; namely, Ritalin® and Nomifensine.

15. Tetrabenazine is a reserpine-like compound, which depletes monoamines and induces sedation, ptosis, and hypothermia. Reversal of these effects by CNS agents is predictive of antidepressant activity and is an indication of occupancy at the NET, although the DAT and 5HT transporters probably play a role as well. NET inhibitors presumably allow enhanced levels of norepinephrine in the synaptic junction which negates the depleting effect of the TBZ (Kelley et al., J. Med. Chem. 39:347-349 (1996); Kunstmann et al.. J. Med. Chem. 30:798-804 (1987); Sulser et al, Psychpharmacologia 8:191-200 (1965); Stille, Arzn. Forsch 14:534-537 (1964)). Thirty minutes after test compounds are administered orally (0.3, 1.0, 3.0, and 10 mg/kg) to male CFI mice (Charles River Breeding Laboratories) weighing 18-25 grams at the time of testing, tetrabenazine (35 mg/kg, i.p.) was administered thirty minutes prior to score time. For the case of the standard CNS acting agent, Ritalin® (methylphenidate), **Graph 1** (below) shows the percent reversal of TBZ-induced effects in mice at the orally administered dose (1.0, 3.0, 10, and 30 mg/kg). Animals were evaluated for antagonism of TBZ-induced exploratory loss and ptosis. Exploratory activity was evaluated by placing the animal in the center of a 5 inch circle. Fifteen seconds were allowed for the animal to move and intersect the perimeter. This is considered antagonism of TBZ and given a score of 0. Failure to leave the circle is regarded as exploratory loss and given a score of 4. An animal was considered to have ptosis if its eyelids are at least 50% closed, and given a score of 4 if completely closed. No closure was given a score of 0. Greater than 95% of the control (vehicle-treated) mice were expected to exhibit exploratory loss and ptosis. Drug activity was calculated as the percentage of mice failing to respond to the TBZ challenge dose. The effects of Ritalin® and amphetamine in the mouse TBZ-induced ptosis model are shown below in **Graph 1** (below).

Graph 1

Methylphenidate Reverses the Sedation Induced by Tetrabenazene (35 mg/kg i.p). in Mice



16. Statistical evaluation: Median effective doses (ED_{50}) and 95 % confidence limits were determined numerically by well-known methods in the art (Thompson, "Use of Moving Averages and Interpolation to Estimate Median-Effective Dose," BACT Reviews 11:115-145 (1947); Litchfield et al., "A Simplified Method of Evaluating Dose-Effect Experiments," J. Pharma. Exp. Therapy 96:99-113 (1949)). The ED_{50} value represents the dosage of the test compound to inhibit the effects of TBZ in at least 50 percent of animals, as measured by pstosis or exploratory activity. Thus, the lower a test compound's ED_{50} value, the greater the efficacy of that test compound to reverse the effects of TBZ. The effective median dose for the reversal of TBZ-induced effects for selected PH-7222 Compounds is summarized in **Table 2** (below).

Table 2

Compound	ED_{50}, mg/kg	
	Ptosis	**Exploratory activity**
Ritalin®	4.0	3.0
Nomifensine	1.0-4.0	4.0
Example 16	2.0	4.0
Example 70	1.2	3.0
Example 74	3.7	5.0
Example 92	0.3	≤0.3
Example 93	≤0.3	1.1

17. Compound **2,** Nomifensine, demonstrated good efficacy and potency for the reversal of TBZ-induced effects in mice. Nomifensine has demonstrated efficacy in clinical studies for the treatment of depression (Brogden et al., Drugs 18(1):1-24 (1979)) and ADHD (Shekim et al., J. Nerv. Ment. Dis. 177: 296 (1989)). The efficacy of this clinical agent is attributed to the potency and selectivity of Nomifensine for blocking norepinephrine and dopamine reuptake at the NET and the DAT. Thus, it would be scientifically reasonable to conclude that a compound with comparable or better efficacy than Nomifensine for reversal of TBZ-induced effects in mice would be a good candidate for the treatment of depression.

18. The results in **Table 2** demonstrate that the PH-7222 Compounds tested using the TBZ assay have either comparable or up to a 12-fold lower ED_{50} value for ptosis and/or exploratory activity than Nomifensine. **Table 2** also demonstrates that the PH-7222 Compounds tested using the TBZ assay have either comparable or up to a 9-fold lower ED_{50} value than Ritalin®. Thus, the results for *in vivo* testing in the TBZ-treated mice demonstrate that the efficacy and potency of the PH-7222 Compounds are comparable to that exhibited by Nomifensine and Ritalin®. Like Nomifensine and Ritalin®, the PH-7222 Compounds demonstrate the ability to penetrate the CNS and act at the relevant monoamine transporters.

19. Based on the results presented herein, it is reasonable to conclude that the compounds of the present invention have utility in methods for treating the various Neurological/Psychological Disorders.

20. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: September 10, 2003 Bruce F. Molino

Bruce F. Molino